THE NEUROMOTOR APPARATUS OF *CHLAMYDODON* SP.

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Introduction.

While working on the life cycle of *Chlamydodon,* a system of fibers around the mouth was found, and, on account of the relation of these fibers to the pharyngeal basket and other organelles, it seemed worth while to make a further study.

As most of the literature on the subject of conductile fibers, especially in the Protozoa, has been reviewed by Taylor (1920), Rees (1922), and Calkins (1926), it seems unnecessary to take up the subject here.

Kofoid applied the term Neuromotor Apparatus to the complex fibrillar system associated with blepharoplasts, parabasal bodies, etc., found in *Giardia.* Other systems of this kind have been described for the flagellates by other workers.

In the ciliates Sharpe (1915) was the first to give the term Neuromotor Apparatus to a complex system of fibrils, having a center or motorium, which he found in *Diplodinium ecaudatum.* Since that time, similar systems have been described for seven other ciliates as follows: Yocom (1913) in *Euplotes patella;* MacDonald (1922) in *Balantidium coli* and *suis;* Rees (1922), *Paramecium caudatum;* Visscher (1925), *Dileptus gigas;* Campbell (1926 and 1927), *Tintinnopsis nucula* and *Favella;* and Picard (1927) *Boveria teredinidi. Euplotes* represents a highly specialized type of Neuromotor Apparatus, *Paramecium caudatum* a generalized type, while that of *Chlamydodon* represents a combination of the two types.

Acknowledgments.

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Calkins, of Columbia University and the Marine Biological Laboratory, for advice and helpful criticism during the progress of the work, and Miss Ruth B. Howland for help that made the work of microdissection possible.

Material and Methods.

The animals used in this investigation were collected from Knowlton's ditch at Woods Hole, Mass. A small portion of algae, chiefly Oscillatoria, from the same ditch, placed in a syracuse dish in Knop's solution one part, distilled water ten parts, furnished an excellent culture medium. By making sub-cultures, the animals were kept in the laboratory at Agnes Scott College for two winters.

Various killing and fixing solutions were used, the best results being obtained with Schaudinn's Fluid, Bouin and strong Flemming. If the material was to be stained with Mallory's connective tissue stain, it was fixed with Zenker's or Picro-mercuric fluid, using the time schedules recommended by Sharp and Yocom. Some of the best preparations were obtained by the use of .3 per cent. hæmotoxylin, long method.

Two methods of embedding were used: (1) by the aid of a LeFevre embedding dish, and (2), by killing a whole culture in a Syracuse dish, and embedding small bits of algæ to which the animals were attached. This was found to be the most satisfactory method, as there was little danger of losing the animals, and the material could be quickly handled; also it gave an abundance of material. Sections were cut from 2–4μ thick. On account of the thickness of *Chlamydodon,* it is impossible to work out the system of fibrils in whole mounts.

All drawings were made with the camera lucida, except Fig. 15, and with the use of 1/7 Leitz oil immersion lens and 12 $\times$ oculars. Details were worked out with 12 $\times$ oculars and a Zeiss 1.5 mm. apochromatic lens. A 250-watt light in a Zeiss microscope lamp was used for illumination.

The Genus *Chlamidodon.*

The genus *Chlamidodon* was named and described by Ehrenberg in the *Proceedings of the Berlin Academy* in 1835. He states that he discovered *Chlamidodon mnemosyne* in the waters of the Baltic Sea near Wismar, Aug. 26, 1834. In 1838, in his Infusionsthierchen als volkomene Organismen, he mentions this form again, and, in addition to the "teeth apparatus" mentioned in his first paper, he describes, a "colorless oval shield," projecting on all sides beyond the body, and covering its body. Stein (1859) differs with Ehrenberg in some details, *e.g.,* he says that he found only eight instead of sixteen trichites in the basket, and he thinks the "oval shield" is just part of the body. Entz (1884) describes *Chlamydodon cyclops* Entz as having fifteen and sixteen trichites in the pharyngeal basket, while Erlanger (1890) figures *Chlamydodon mnemosyne* with sixteen. Stein changed the spelling from *Clamidodon* to *Chlamydodon.*

Ehrenberg placed the genus in the family "Euplota"; Stein placed it in the family "Chlamydodonta."

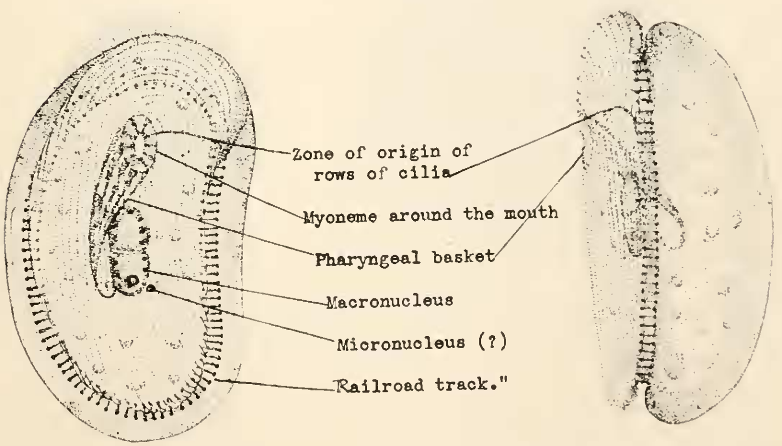


FIG. 1 *A.* *Chlamydodon* sp. (Probably a variation of *mnemosyne.*) Ventral view.

FIG. 1 *B.* Side view.

The form most common at Woods Hole is probably a variation of *Chlamydodon mnemosyne,* though it differs very much from Erlanger's description of that species. There is another species of *Chlamydodon,* found at Woods Hole, which the writer has seen only twice, and only isolated specimens. It has a very short pharyngeal basket situated at the extreme anterior end. It differs from all described species in the character of its cilia.

The species which was used as a basis for the present study, Fig. 1, measures from 60–70μ in length, and 40–45μ in breadth. Like all of the species, it is ciliated on the ventral side only, the anterior cilia being much longer than those on the other parts of

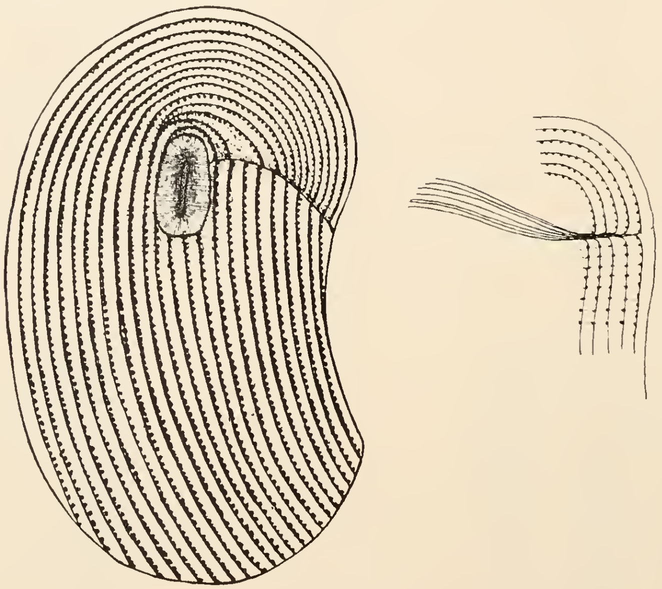


Fig. 2 *A*. Arrangement of cilia and fibers traversing the circular myoneme.
Fig. 2 *B*. Fan of fibers from cilia just before they join motorium. Delicate cross fibers from basal bodies of cilia.

the body. The cilia are very fine, and arranged in rows set close together. To get the true arrangement of cilia, the ventral surface has to be removed and flattened out. This is possible only when the "railroad track" has been cut, as this structure bounds the rows of cilia, and, at the anterior end pulls a part of the ventral surface over on the dorsal side, Fig. 1 *B* and Fig. 4. The anterior cilia take their origin from a zone on the right-hand side (ventral side up), extend around the anterior end in a half circle, thence to the posterior boundary of the "railroad track" in a somewhat curved line, Fig. 2. The cilia below the zone on the right-hand side, take their origin as said zone, and extend in curved lines to the posterior boundary of the railroad track. The three central rows begin at the posterior end of the myoneme surrounding the mouth, and extend to the posterior end of the " railroad track " in the same manner as the other rows, Fig. 2. Fig. 1 *B* shows the manner in which the " railroad track " bounds the rows of cilia.

FIG. 3. Sections showing various changes in shape of the " railroad track."
FIG. 4. Sections of entire animal showing changes in the shape of the " railroad track," and changes in the shape of the animal.

The peciliar organelle known as the " railroad track " is a band of trichites encircling the animal in the manner shown in Fig. 1 *B*. In some of the older papers, the exact location of this band is a matter of discussion. We were able to pierce the dorsal wall of the animal with a micro pipette, and blow out the cell contents, including the pharyngeal basket; nothing was left except the body wall, almost entire, and the " railroad track." Its position was unchanged. It is covered with a thin pellicle, and fastened tightly to it. With the micro dissection needles, the track was cut out and pulled apart. While it was easier to tear apart where it was thin, we could not duplicate the effect shown by Erlanger. The figure in Erlanger's paper seems to show the parts of the " railroad track " easily separable into round masses of protoplasm, each having a trichite in the center.

If the animal is viewed from the ventral side, the structure looks flat, and the trichites arranged after the manner of crossties, hence the name " railroad track." A side view of whole mounts, easily obtainable, and sections, shows that the ordinary shape of this structure is half a circle. The organelle has the power of changing its shape. This is readily shown in sections; it may be almost completely closed, or wide open, Figs. 3 and 4. After this observation was made, a careful study of the living animal showed that when the lip was bent back very far the structure was closed, forming a complete ring. The anterior end was closed during ingestion of food.

The ends of the trichites seem to be heavier than the middle portion, and between each two trichites there is a thin portion with a place in the center easily pulled apart. When the organelle is cut and straightened out, it is seen that the trichite is thickened and the thin portion reduced, the end view reminding one of an accordion, Fig. 17.

FIG. 5. The pharyngeal basket of *Chlamydodon* sp., with circular myoneme which covers it removed.

FIG. 6. Side view, showing relation of basket to myoneme.

The band or "railroad track" is not interrupted, as shown in Erlanger's figures of *Chlamydodon mnemosyne*. Just before division, a break near the center may be observed.

The pharyngeal basket is very large in proportion to the size of the animal. It is very different from the basket described by Erlanger, and figured in Doflein, p. 54. There are ten heavy trichites, the anterior ends being expanded, and the trichites showing distinctly. About half way down, the trichites seem to fuse, their identity becoming lost as the basket narrows, resembling *Chilodon uncinatus* in this respect. The posterior end has a small filament wound to the right (ventral view). At the anterior end, each trichite has a sort of cap fitted to it somewhat after the manner of a hinge, Figs. 5 and 6, and extending to the mouth opening. These caps are in the shape of a triangle, and form a lid. This lid is, in turn, covered by a circular myoneme, like a sphincter muscle, in the center of which is the mouth opening. As Figs.

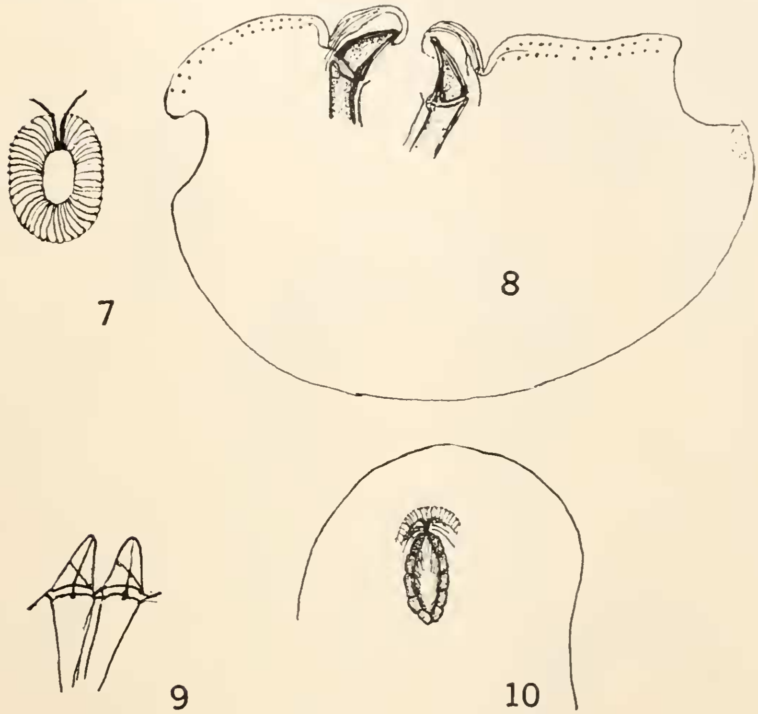
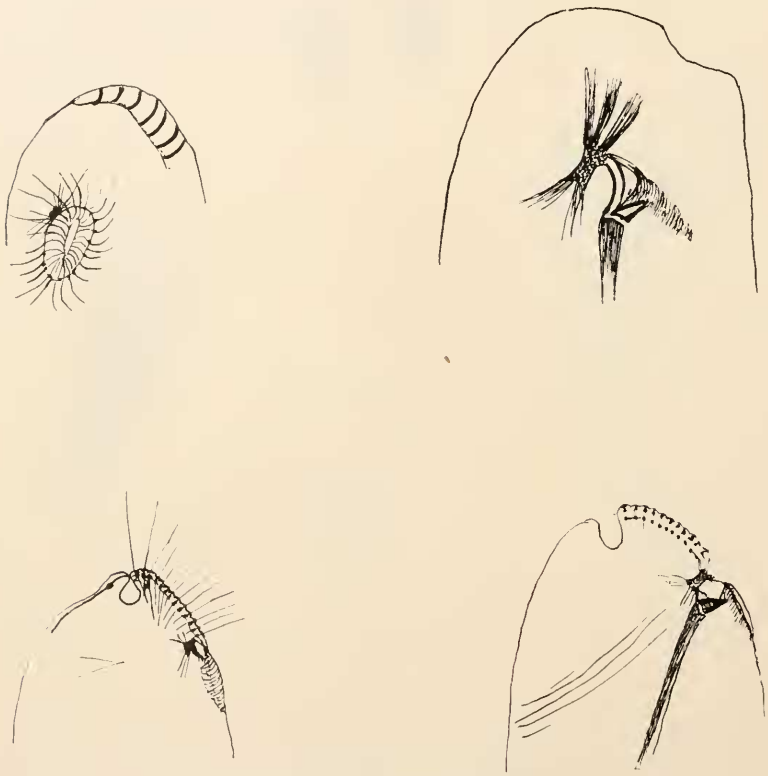


Fig. 7. Circular myoneme surrounding the mouth, with traversing fibers.
Fig. 8. Tangential section through mouth region, showing relation of basket to myoneme and fibers.
Fig. 9. Fibers in caps of trichites and top of trichites.
Fig. 10. Caps removed from the trichites of the basket. Note fan of fibers.

6 and 8 show, the edges of the mouth opening in this myoneme fit under the points of the triangles. It is suggested that the triangles are pulled back by the myoneme, and so opens the mouth.

The macronucleus is divided into two parts, the anterior part taking the stain more lightly than the posterior half. In the resting stage, the granules in the anterior half form a complete horseshoe. These granules change in size and position as cell division proceeds. There are, in addition, many small granules. The posterior half has a large division center, and many other smaller granules. Between the two halves there is a split, or querspalt. Often a granule is found in this split, and it has been observed to divide. The two granules migrate into the cytoplasm. It has not



FIGS. 11, 12, 13, 14. The Motorium, and its relation to other structures.

been possible to follow their further history. In division, the split disappears, the division center moves up the center of the nucleus, pulls out and divides. All of the stages of division have not yet been worked out.

The position of the micronucleus is uncertain. Entz pictures it on the side of the nucleus in *Chlamydodon cyclops,* and Erlanger shows it imbedded in the anterior end of the macronucleus. It is quite small, and some of my preparations show it at the posterior end of the macronucleus, and some at the side. It cannot be seen in all preparations.

The Neuromotor Apparatus

The first part of the neuromotor apparatus to be observed was a system of fibers around the mouth or oral opening, Figs. 2, 6, 7. As described above, the pharyngeal basket in *Chlamydodon* is very large and heavy, and the circular myoneme around the mouth is seen to be traversed by many fine fibers, with a small granule for

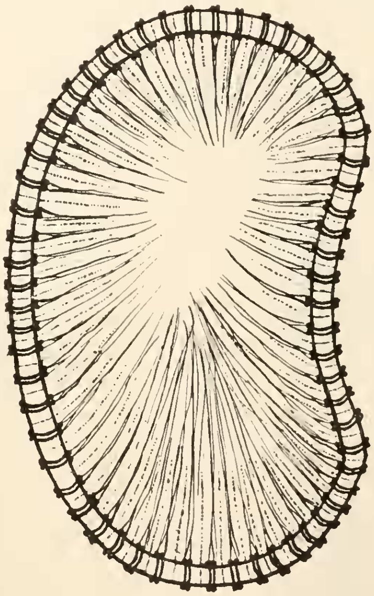


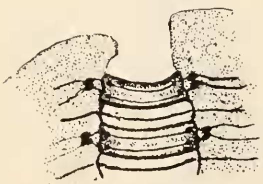
Fig. 15. Diagrammatic representation of the relation of fibers to the "railroad track."

each one on the edge of the myoneme, Fig. 7. In only one or two favorable preparations could it be seen that these fibers run out into the cytoplasm in the general direction of the "railroad track," Fig. 11. With the aid of micro dissection needles, it is possible to lift off the ventral surface of the animal. If this is then properly stained, it is seen that the myoneme around the mouth is continuous with the body wall, but much thicker. The fibers from the myoneme extend under the rows of cilia. They are very fine, and it is impossible to trace them far.

Fibers run from one trichite to the next, and there are cross fibers on each of the caps, Fig. 9. These fibers all come together in two heavy strands, joining other fibers around the mouth, and connecting the motorium at the lower right-hand corner, Figs. 12 and 13.

The basket seems to be lined with a very thin membrane, and when the caps are removed from the trichites, fibers can be seen in this lining, Fig. 10.

The motorium is just below the anterior end of the basket, and is placed a little slantingly, Figs. 11, 12, and 13. It is a bilobed mass, which stains with hæmotoxylin. It, and the other fibers in the system, stain bright red with Mallory's connective tissue stain.



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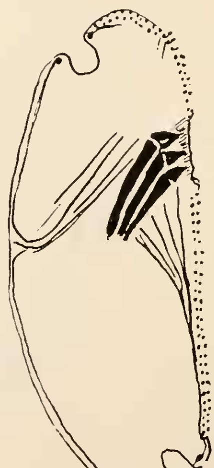

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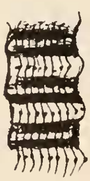

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Fig. 16. Portion of the "railroad track" greatly enlarged.

Fig. 17. Portion of the "railroad track" dissected out and flattened. Shows peripheral fibers.

Fig. 18. Section showing dorsal and ventral fibers from the "railroad track."

It is impossible to see the motorium in the whole mounts. On account of its proximity to the pharyngeal basket, it was overlooked for a long time, for the basket is heavy, and when destaining is carried on long enough to differentiate it, the motorium is destained. In sections, however, the structure is easy to see. With the aid of microdissection needles, the basket can be dragged out whole, and the motorium is sometimes pulled out with it. It is then seen to be a refringent body, while in stained sections its general structure appears granular. Fig. 12 shows the fans of fibers joining the motorium.

The peculiar organelle known sometimes as the " striped band " or " railroad track " mentioned above, has a very complex system of fibers connected with it. Sections show the fibers very well, but their paths are hard to trace on the ventral side on account of the presence of the cilia.

At each end of each trichite, there is a plate or mass, Fig. 16. In sections this mass appears single, Figs. 13 and 18; when viewed from the ventral side of the animal, it appears bilobed or double, Fig. 16. There is a possibility that when the granules were observed from the ventral surface, they were in the process of division, but, when they were seen at all from this angle, they appeared double. The presence of fine rows of cilia on the ventral surface obscures everything; on the dorsal surface the body is much curved in the region of the railroad track, making it impossible to see the granules or masses so close to the organelle. The fibers that go through the trichites are connected with these masses. Fig. 15, in a somewhat diagramatic way, shows the relation of one set of ventral fibers with the masses and the trichites. There are two sets of fibers, one set that enters the trichites, and one set runs through the thinner portion of the "railroad track," Fig. 15. These fibers are from two levels. When the mouth is open, during the ingestion of food, the railroad track changes its shape from a half circle to a closed circle, Fig. 4. From the evidence of the sections, and the observations upon the living material, it is reasonable to suppose that one set of these fibers is connected with the myoneme around the mouth. The presence of ciliary lines and fibers make it almost impossible to trace these fine lines.

The dorsal fibers extend only a short distance underneath the pellicle, then they turn and go in the direction of the motorium.

Only two preparations showed with any clearness the fans of fibers joining the motorium. The basal bodies of the cilia are connected both by longitudinal and cross fibers, Fig. 2 *B*. The longitudinal fibers of the cilia turn in at the zone of origin of the cilia, and connect with the motorium at the anterior end, Fig. 12. This end of the motorium also receives the fibers from the ventral surface of posterior end of the animal. Fig. 15, and some fibers from the mouth region. Figs. 13 and 14. The dorsal fibers join the motorium at the posterior end.

As described above, between each two trichites, there is a thinner portion, and in the center of this is a series of small granules. The granules are connected with fibers which pass over the trichites to the next set of granules, Fig. 17. In some sections, the accordion like arrangement is more pronounced than others, suggesting that there is power of movement, a sort of folding of the trichites. No observations on the living material settled this point, but further evidence of the possibility of the movement suggested lies in the fact that the trichites are sometimes closer together than at other times.

Microdissection.

After the location of the motorium, twenty-five animals were successfully cut, freehand, with the aid of micro-dissection needles given to me by Dr. Robt. W. Chambers of Cornell Medical College. Later, Miss Howland, of New York University, operated upon several with the Chambers micro-dissection apparatus.

The cilia of *Chlamydodon* are fine, the anterior ones being longer and easier to observe than the posterior ones. The motorium cannot be seen in unstained specimens but its position with relation to the basket is known, so that it is quite simple to destroy it. If the motorium be destroyed, there is a marked disturbance in the action of the cilia, in no way comparable to the disturbance of the cilia if other parts of the body are injured. The cilia still have their wave like motion, however, and this is to be expected when one takes into consideration the relation of connecting fibers of the cilia, Fig. 2 *A* and *B*. However, the cilia do not reverse after the destruction of the motorium as is usual in intact animals. Isolated pieces behave the same way, as has been observed by Jennings and Jamieson (1902), and by Rees (1922). Animals without conspicuous motile organs are not favorable material for the study of the coördination by means of microdissection.

Summary.

A description of *Chlamydodon*, probably a variation of the species mnemosyne, found in brackish water at Woods Hole, is presented.

There is a complex neuromotor apparatus, including a coördinating center, and systems of fibers connected with cilia, the mouth opening, the phryngeal basket, and the " railroad track."

The connection of the fibers with the organelles and the central mass, or motorium, the behavior of the animal after the destruction of the motorium, seem to suggest a coördinating function for the system.

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